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THE CROSS-TRANSMISSION POTENTIAL OF *CRYPTOSPORIDIUM* SPP.

OVERVIEW

Since its discovery in mice in 1907, the sporozoan parasite *Cryptosporidium* has been detected in a wide range of host animals. However, the pathogenic significance of the parasite was not established until the early 1970's when infections were implicated as the causative agent of diarrhoea in calves and man. Since then, numerous reports have described clinical cryptosporidiosis involving diarrhoea in mammals, respiratory signs in birds and gastritis in reptiles and fish.

Early reports assumed the parasite to be host-specific and some 20 *Cryptosporidium* spp. were named on the basis of host occurrence. However, recent cross-transmission studies revealed that infections in mammals may be transmitted to other mammalian species (including man) thereby establishing the zoonotic potential of the parasite. In contrast, cross-transmission studies involving donor and recipient hosts belonging to different vertebrate classes have largely been unsuccessful therefore it was suggested that separate *Cryptosporidium* spp. occur in the different vertebrate classes. This level of host specificity remains to be confirmed by further experimental studies. Nonetheless, six parasite species are currently regarded as valid;

- *C. muris* and *C. parvum* in mammals,
- *C. meleagridis* and *C. baileyi* in birds,
- *C. serpentis* in reptiles and
- *C. nasorum* in fish.

Recent morphological and isoenzyme characterization studies have detected considerable structural and genetic diversity among *Cryptosporidium* isolates from hosts belonging to the same and different vertebrate classes. These results suggest that multiple cryptic species may occur not only between different host species but also within the same host species. The taxonomy of *Cryptosporidium* spp. therefore remains to be resolved and the cross-transmission potential of each species must be carefully determined to ascertain the risks posed to both human and animal populations.

HISTORICAL BACKGROUND

Developmental stages of a novel protozoan parasite were described in the gastric glands of laboratory mice by Tyzzer in 1907 and 1910. The parasites were small in size and were attached to the surface of the epithelial cells by knob-like projections. Both asexual and sexual developmental stages were described culminating in the formation of spores (i.e. oocysts). The oocysts were unique in appearance in that they contained four sporozoites which were not enclosed within secondary spores (i.e. sporocysts). The parasites were considered to be similar to other coccidian sporozoan parasites and it was proposed that a new genus be erected and named *Cryptosporidium* (meaning hidden sporocysts). The species occurring in the gastric glands of mice was named *C. muris*.

Similar parasites were later detected in the small intestines of mice by Tyzzer in 1912. The parasites were smaller in size than *C. muris* and their development was confined to the epithelium of the small intestine. They were considered to represent a separate parasite species on the basis of their size and location and were named *C. parvum*. Organisms similar in morphology to *C. parvum* were later described in association with the caecal epithelia of chickens by Tyzzer in 1929.

Despite several further reports on the occurrence of the parasites, their pathogenic significance was unclear until 1955 when Slavin described infections by a new species (named *C. meleagridis*) in the intestines of turkeys in association with acute clinical disease characterized by severe diarrhoea and low mortalities. The pathogenicity of the parasite was consequently established, at least in birds.

In 1971 and 1974, *Cryptosporidium* parasites were detected in the intestines of calves which had histories of chronic diarrhoea. Veterinary interest was aroused and further case studies on diarrhoeic calves revealed *Cryptosporidium* infections to be associated with acute and chronic clinical disease in the absence of concomitant infections by other enteropathogens such as bacteria and viruses. The parasite has since been found to be a common enteropathogen in a variety of neonatal domestic animals.

In 1976, two cases of *Cryptosporidium* infection were reported in human patients presenting with severe watery diarrhoea. Additional cases were reported intermittently

over the next few years; many involving immuno-compromised individuals who had congenital immunological disorders or were undergoing immuno-suppressive therapy. Further medical interest was generated in 1982 when infections were related to several mortalities in HIV-infected patients with acquired immuno-deficiency syndrome (AIDS). In the same year, an outbreak of cryptosporidiosis was reported in healthy immuno-competent individuals who had been in close contact with infected calves thereby establishing the potential for zoonotic transmission.

Since then, *Cryptosporidium* infections have been associated with mild to severe gastroenteritis in numerous immuno-compromised and immuno-competent individuals throughout the world. Asymptomatic infections have also been detected with increased frequency in healthy individuals. The parasite is more prevalent and widespread than previously thought and epidemiological studies frequently implicate animals as sources of human infection although the cross-transmission potential of most isolates is undetermined.

PARASITE MORPHOLOGY

Early studies on infections in animals were based on the light microscopic detection of parasites in histological sections of tissues collected at post-mortem. Early studies on humans were based on tissue biopsy samples collected from patients exhibiting clinical signs of disease. *Cryptosporidium* infections were characterized by the presence of small basophilic bodies in close association with the luminal surface of the intestinal epithelium.

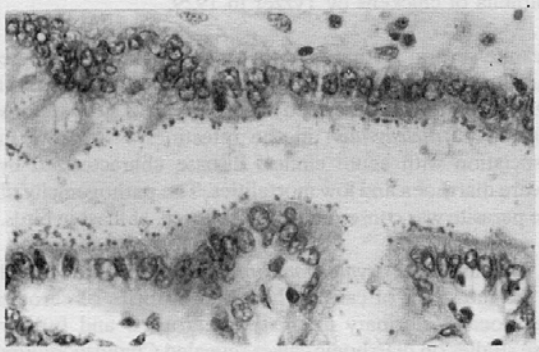

Fig. 1. *Cryptosporidium* developmental stages lining the epithelium of the small intestine from a goat (*Capra hircus*). Light micrograph, H&E, x 250.

Parasite developmental stages may be so prolific in heavily infected tissues that they give the epithelial surface a rough granular appearance. However, the parasites are usually not uniformly distributed over the epithelium but are localized to focal aggregates or small pockets of infection. Most infections have been detected in close association with the intestinal epithelia but organisms have also been detected in other locations; including the stomach, gall bladder, bile ducts, pancreatic ducts and respiratory tract of mammals; the urinary tract, respiratory tract, conjunctival sacs, bursa and cloaca of birds; and the stomachs of fish. Most infections in reptiles have been confined to the stomach although some have been detected in the cloaca of lizards.

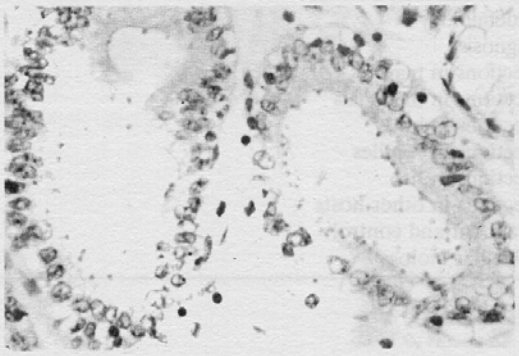

Fig. 2. Developmental stages of *Cryptosporidium* located in the gastric pits of a taipan (*Oxyuranus scutellatus*). Light micrograph, H&E, x 250.

In all cases, the parasites were located within the brush border or microvillous layer of the epithelial cells. They are spherical to elliptical in shape measuring from 2-6 μm in diameter. The parasites appear to protrude from the cell surface and it was originally thought that they were extracellular and attached to the external surface of the host cell membrane. However, electron microscopic studies have revealed that they are intracellular and are enclosed within parasitophorous vacuoles formed by a continuous covering of microvillous membranes.

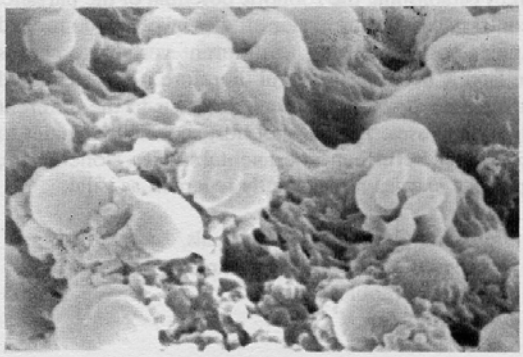

Fig. 3. Endogenous stages of *Cryptosporidium* in the trachea of a common quail *Coturnix coturnix*. Scanning electron micrograph, x 1,250.

The parasites are located beneath the host cell membrane but they are separated from the host cell cytoplasm by the parasitophorous vacuole membrane. They are described as being intracellular but extracytoplasmic. Other sporozoan parasites also develop within parasitophorous vacuoles but they are usually located deep within the cytoplasm next to the host cell nucleus. The peripheral location of the endogenous developmental stages of *Cryptosporidium* in the microvillous layer of epithelial cells is unique.

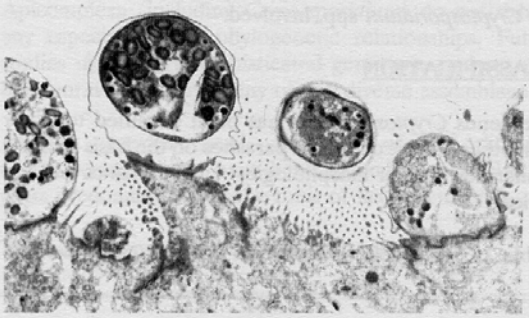

Fig. 4. Endogenous developmental stages of *Cryptosporidium* located in quail trachea. Transmission electron micrograph, x 6,000.

The parasites also contain a unique 'attachment' or 'feeder' organelle which is prominent at the base of each parasitophorous vacuole. The parasite pellicle is repeatedly folded to form a comb-like lamella which is closely associated with a dense adhesion zone formed by fusion of the outer microvillous membrane and the epithelial plasma membrane. This organelle is thought to facilitate the uptake of nutrients by the parasite from the host cell.

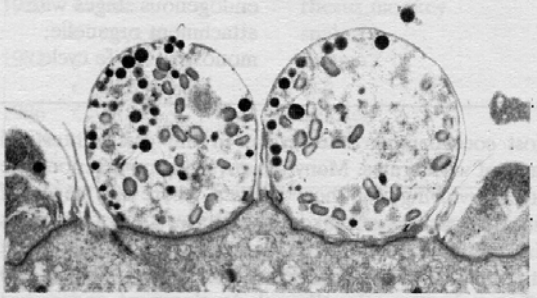

Fig. 5. *Cryptosporidium* macrogametocytes in quail bursa. Transmission electron micrograph, x 8,000.

Both uninucleate and multinucleate stages of the parasite may be found which represent different asexual and sexual multiplicative forms. The final product of sexual reproduction is the formation of an oocyst which is passed in the faeces of the host as the exogenous infective stage. The structural configuration of mature *Cryptosporidium* oocysts is unique in that they contain four sporozoites which are not enclosed within a sporocyst. Oocysts recovered from different host species may vary in size (ranging from 4.5-7.9 μm in length by 4.2-6.5 μm in width) and they may be ovoid to elliptical in shape (length/width shape indices ranging from 1.1-1.4).

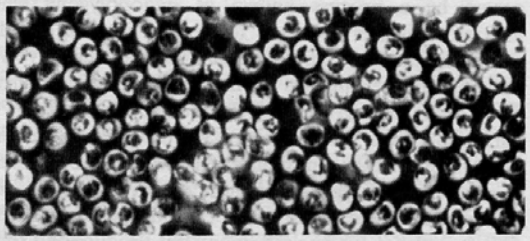

Fig. 6. *Cryptosporidium* oocysts harvested from quail faeces. Light micrograph, phase-contrast, x 1,250.

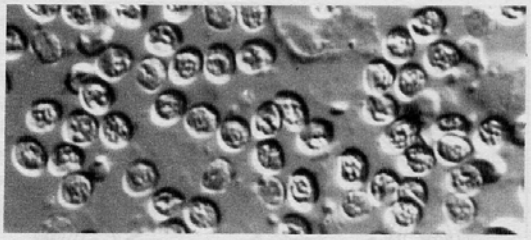

Fig. 7. *Cryptosporidium* oocysts harvested from taipan faeces. Light micrograph, Nomarski interference-contrast, x 1,500.

All mature developmental stages of *Cryptosporidium* (i.e. sporozoites and merozoites) exhibit many ultrastructural characteristics common to other enteric sporozoan parasites. The zoites are bounded by a three-layered pellicle and they contain an apical complex, micronemes, electron dense bodies and subpellicular microtubules. However, certain other organelles appear to be absent (e.g. conoid, rhoptries and micropores).

LIFE CYCLE

Light and electron microscopic studies performed on naturally and experimentally infected hosts have revealed that *Cryptosporidium* spp. have a monoxenous life cycle (where all stages of development occur within one host).

Oocysts ingested by host animals excyst in the gastro-intestinal tract releasing the infective sporozoites. Excystation has been reported to be triggered by various factors including reducing conditions, carbon dioxide, temperature, pancreatic enzymes and bile salts.

Sporozoites escape through a slit-like opening which is created at one end of the oocyst by dissolution of a special suture in the oocyst wall. The freed sporozoites attach to epithelial cells where they become enclosed within parasitophorous vacuoles and develop attachment organelles.

The parasites then undergo asexual proliferation by merogony (formerly called schizogony). Cell division occurs by a process known as endopolygeny where multiple daughter cells are formed by internal budding within the mother cell. Sequential development involving two types of meronts has been described. Type I meronts form eight merozoites which are liberated from the parasitophorous vacuole when mature. The merozoites then invade other epithelial cells where they undergo another cycle of type I merogony or develop into type II meronts. The type II meronts form four merozoites which do not undergo further merogony but produce the sexual reproductive stages (called gamonts).

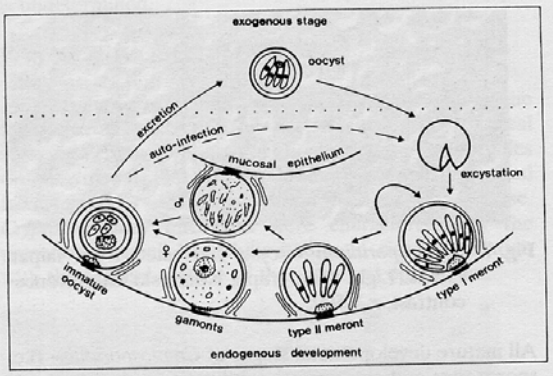

Fig. 9. Generalized *Cryptosporidium* life cycle.

Sexual reproduction occurs by gametogony and both male and female gamonts are formed (known as microgamonts and macrogamonts respectively). Microgamonts develop into microgametocytes which contain 16 microgametes. Macrogamonts develop into uninucleate macrogametocytes which are fertilized by mature microgametes. The resultant zygotes undergo further asexual development (sporogony) leading to the production of sporulated oocysts containing four sporozoites.

Most oocysts are excreted with host faeces and infections in other hosts become established following oocyst ingestion. Some oocysts, however, have been reported to excyst within the same host animal leading to a new cycle of development (auto-infection). These oocysts are thought to be thin-walled in comparison to the thick-walled oocysts which are excreted from the host.

The entire life cycle of the parasite may be completed in as little as 2 days in many hosts and infections may be short-lived or may persist for several months. The period of time between infection and oocyst excretion (i.e. the prepatent period) ranges from 2 to 14 days in most domestic animal species. The duration of oocyst excretion (i.e. the patent period) has been found to be quite variable within and between different host species ranging from several days to several months. Many factors may influence the longevity of infections but the majority appear to involve the immunocompetency of the host and the *Cryptosporidium* spp. involved.

CLASSIFICATION

The genus *Cryptosporidium* has been classified together with other enteric coccidian parasites on the basis of many similarities in their morphological characteristics and life cycles.

Table 1. Taxonomic classification of *Cryptosporidium*

Phylum:	Apicomplexa	Apical complex present; all species parasitic.
Class:	Sporozoasida	Reproduction asexual and sexual; oocysts or 'spores' produced.
Subclass:	Coccidiasina	Life cycle generally involves merogony, gametogony and sporogony; small gamonts.
Order:	Eucoccidiorida	Merogony or 'schizogony' present.
Suborder:	Eimeriorina	Macro- and micro-gamonts develop independently; non-motile zygote.
Family:	Cryptosporidiidae	Oocysts contain four naked sporozoites (no sporocysts); endogenous stages with attachment organelle; monoxenous life cycle.

Most coccidian parasites occur in the gastro-intestinal tracts of vertebrates. Many genera have monoxenous life cycles involving one host species where all stages of parasite development take place. Better known examples of these 'true' coccidia include *Eimeria* and *Isospora*. Other genera have heteroxenous life cycles involving two different host species. Sexual development and oocyst formation occurs in one host species (known as definitive hosts) whereas asexual extra-intestinal development takes place in another host species (known as intermediate hosts). Genera belonging to these 'tissue cyst-forming' coccidia include *Toxoplasma*, *Hammondia*, *Besnoitia*, *Sarcocystis* and *Frenkelia*.

Other parasites classified in the same order (but different suborders) include malarial and haemosporidian parasites (such as *Plasmodium* and *Haemoproteus*) and adeleorin coccidia (such as *Klossiella* and *Haemogregarina*). The taxonomic classification of these different parasites is currently based on multiple phenotypic characters and the phylum Apicomplexa was recently created to accommodate those possessing an apical complex at some stage of their development. However, recent molecular biological studies using small subunit ribosomal RNA sequence analyses have indicated that many members of the phylum Apicomplexa (including *Cryptosporidium*) do not exhibit any especially close phylogenetic relationships. Future studies using more sophisticated genotypic analyses may help unravel the phylogeny of this diverse assemblage.

At the species level, a total of 21 different *Cryptosporidium* spp. have been named over the last 70 years predominantly on the basis of host occurrence.

Table 2. Named species of *Cryptosporidium*

1910	*C. muris*	mouse
1912	*C. parvum*	mouse
1925	*C. crotali* ?	rattlesnake
1938	*C. vulpis* ?	fox
1955	*C. meleagridis*	turkey
1961	*C. tyzzeri*	chicken
1968	*C. lampropeltis* ?	king snake
1969	*C. ameivae* ?	lizard
	C. ctenosauris ?	lizard
1971	*C. wrairi*	guinea pig
1974	*C. agni*	sheep
	C. anserinum	goose
	C. bovis	ox
1979	*C. cuniculus*	rabbit
	C. felis	cat
1980	*C. rhesi*	rhesus monkey
	C. serpentis	snakes
1981	*C. garnhami*	human
	C. nasorum	fish
1986	*C. baileyi*	chicken
	C. curyi ??	cat

However, not all of the named species are considered to be valid. Parasites originally described from rattlesnakes, foxes, king snakes and lizards are now thought to have been the free sporocyst stages of various *Sarcocystis* spp. rather than the oocysts of *Cryptosporidium* spp. Furthermore, the second report of a species in cats described oocysts approximately 5-10 times larger than those of any other *Cryptosporidium* species. More recently, the validity of many other species has been called into question due to the results of cross-transmission studies.

CROSS-TRANSMISSION STUDIES

Most original reports assumed the parasites to be host-specific with transmission being confined to individual host species. New parasite species were therefore named for each new host found to be infected. Multiple parasite species differing in oocyst morphology and sites of infection were also thought to occur within several individual host species (notably mice and domestic poultry).

However, early cross transmission studies performed in the 1980's indicated that infections in domestic animals were readily transmissible to other domestic and laboratory animal species. The apparent lack of host-specificity prompted the suggestion that *Cryptosporidium* should be considered a monotypic (or single-species) genus. Variations observed in the infectivities of different isolates were thought to have resulted from parasite strain differences or from variations in host susceptibility due to age-related or immune-mediated resistance.

Shortly thereafter, the first immunoserological study reported the detection of specific antibodies in human and animal sera using parasite antigens derived from a single source (infected tissues from one lamb). The apparent lack of immunological specificity was taken as further evidence in support of a monotypic genus. Accepting this postulate would mean that all *Cryptosporidium* isolates should be regarded as potentially zoonotic.

In 1984, a review of all previous cross-transmission experiments noted that they had been performed exclusively using *Cryptosporidium* isolates from mammals. The majority reported the successful transmission of isolates from one mammalian species to another; including isolates derived from humans. Zoonotic transmission from domestic animals to humans had previously been demonstrated when several animal attendants contracted clinical cryptosporidiosis after handling infected calves. Several studies had attempted to transmit infections from mammals to birds but most were unsuccessful whereas no attempts had been made to transmit isolates from birds, reptiles or fish to any other species other than the one of origin.

On the basis of these cross-transmission studies, it was proposed that four species of *Cryptosporidium* should be considered valid; one for each vertebrate class. The four proposed species were:

- *C. muris* in mammals,
- *C. meleagridis* in birds,
- *C. crotali* in reptiles (species subsequently declared invalid and replaced by *C. serpentis*), and
- *C. nasorum* in fish.

The proposal to consolidate all named *Cryptosporidium* spp. into four species specific for different vertebrate classes prompted further studies on host specificity and parasite morphology.

Table 3. Successful cross-transmission studies.

Donor	Recipients
Mammals:	
human	→ human, calf, lamb, goat, pig, dog, cat, guinea pig, rat, mouse, chicken
calf	→ human, calf, lamb, goat, deer, foal, pig, dog, cat, guinea pig, hamster, rabbit, rat, mouse, opossum, chicken
mouse	→ calf, goat, pig, dog, cat, rabbit, guinea pig, rat, mouse
rat	→ rat, lamb, cat, rabbit, guinea pig
guinea pig	→ guinea pig, lamb, calf, mouse
lamb	→ lamb, pig, rat, mouse
goat	→ goat, mouse
pig	→ pig, calf
deer	→ mouse
dog	→ mouse
cat	→ cat
Birds:	
chicken	→ chicken, turkey, goose, duck, pheasant, partridge, guinea fowl, calf
quail	→ chicken, dog, cat, rabbit, guinea pig, mouse
turkey	→ chicken, duck
pheasant	→ chicken

Table 4. Unsuccessful cross-transmission studies.

Donor	Recipients
Mammals:	
guinea pig	- rabbit, rat, mouse, chicken, turkey
cat	- mouse, guinea pig
human	- chicken, mouse
calf	- chicken, fish
mouse	- rat
Birds:	
chicken	- quail, calf, goat, pig, guinea pig, hamster, gerbil, rat, mouse
quail	- chicken, mouse
pheasant	- mouse
Reptiles:	
snake	- mouse
lizard	- mouse

The majority of studies involving mammal-to-mammal or bird-to-bird transmission have been successful whereas most mammal-to-bird or bird-to-mammal studies have failed; thereby providing some support for the concept of vertebrate class specificity. However, negative cross-transmission results must be viewed with caution because a variety of other factors may affect their outcome; including the viability of the inoculum, the route of inoculation and the age and immune status of the recipient host. In fact, several mammalian isolates were found to produce light subclinical infections in birds thereby suggesting some lack of host specificity.

Comparative morphological studies have also revealed considerable heterogeneity between different isolates. Two parasite species were originally described in mice on the basis of differences in their sizes and sites of infection. Such differences have subsequently been confirmed by studies in mice and other mammals. It was therefore proposed that the species *C. muris* be resurrected as a valid species in mammals. *C. muris* differs from *C. parvum* in that its oocysts are larger and endogenous development occurs in the stomach rather than the small intestines.

The characteristics of most infections reported in mammals, however, are consistent with those of *C. parvum*. In retrospect, it is probable that most cross-transmission studies performed with isolates from mammals involved *C. parvum*. Natural infections by *C. muris* have only been detected in a small range of mammalian hosts and comprehensive cross-transmission studies have not yet been performed. Nonetheless, a strain of *C. muris* originally isolated from rats has recently been transmitted to chickens and back to various mammals suggesting infections may not be restricted to mammals.

Another species was also described in birds on the basis of differences in structure and sites of infection. *C. baileyi* from chickens was distinguished from *C. meleagridis* from turkeys because the oocysts were larger, they were less infective to other bird species and endogenous development occurred in the respiratory tract and bursa rather than the small intestines. Additional species may also occur in birds because several isolates have recently been recovered which do not conform to either species description. The extent of their host ranges remains to be determined but infections may not be restricted to avian hosts. Oocysts resembling *C. baileyi* were recently recovered from a human patient and were successfully transmitted to chickens but not to mice. This result indicates that some avian isolates must be regarded as potentially zoonotic.

Considerable morphological variation has been detected among isolates from reptiles. At least five separate groups have been identified on the basis of differences in oocyst

size and shape suggesting the existence of multiple parasite species. However, cross-transmission studies involving these isolates have not yet been performed.

Most early reports of infections in fish provided little morphological detail but recent ultrastructural studies have revealed isolates from cichlids to possess several unique characteristics. Endogenous developmental stages were covered by an unusual microvillous membrane with prominent protrusions whereas the oocysts were enclosed within degenerating host cells. Similar characteristics have not previously been recorded for any *Cryptosporidium* spp. No studies have yet been conducted to determine the host specificities of any isolates from fish.

At present, a variety of characters are used to differentiate *Cryptosporidium* spp. Six species are currently considered to be valid on the basis of apparent host specificity, parasite morphology and site of infection.

Table 5. Current classification of *Cryptosporidium* spp.

Parasite species	Vertebrate host class	Usual site of infection	Oocyst length (μm)	Oocyst width (μm)
C. parvum	mammals	intestines	4.5-5.4 ($\bar{x}$=5.0)	4.2-5.0 ($\bar{x}$=4.5)
C. muris	mammals	stomach	6.6-7.9 ($\bar{x}$=7.4)	5.3-6.5 ($\bar{x}$=5.6)
C. meleagridis	birds	intestines	4.5-6.0 ($\bar{x}$=5.2)	4.2-5.3 ($\bar{x}$=4.6)
C. baileyi	birds	respiratory tract, bursa	5.6-6.3 ($\bar{x}$=6.2)	4.5-4.8 ($\bar{x}$=4.6)
C. serpentis	reptiles	stomach	5.6-6.6 ($\bar{x}$=6.2)	4.8-5.6 ($\bar{x}$=5.3)
C. nasorum	fish	intestines, stomach	3.5-4.7 ($\bar{x}$=4.3)	2.5-4.0 ($\bar{x}$=3.2)

However, further studies are required to confirm the validity of these characters for taxonomic and diagnostic purposes. Many isolates have been detected which do not conform to any species description and the host specificity of most isolates has not been determined. Some cross-transmission between birds and mammals has previously been reported and our studies have suggested that cross-transmission between reptiles and mammals may occur. It is therefore advisable to treat all *Cryptosporidium* isolates with caution until their cross-transmission potential can be established.

EPIDEMIOLOGY

Infections by *Cryptosporidium* were originally thought to occur infrequently and to be asymptomatic in a limited number of host species. However, recent studies have revealed the parasites to be more prevalent and pathogenic than previously thought. Over 120 different host species have been identified and infections have been detected world-wide in over 50 countries ranging in location from tropical to temperate zones.

The prevalence of infections within given animal or human populations is difficult to determine with any degree of accuracy. Early studies were limited to individual hosts succumbing to disease or experiencing diarrhoea severe enough to have warranted medical or veterinary attention. Numerous coprological surveys have since been performed on samples submitted to diagnostic laboratories but such studies usually only accounted for clinical infections. Recent coprological surveys have included samples from asymptomatic individuals but many infections may not have been detected because oocysts were excreted sporadically or in low numbers. In fact, various immunoserological techniques used to provide presumptive evidence of prior exposure to infections have indicated that infections may be more common than suggested by coprological surveys. Some seroprevalence results have even ranged as high as 50% within the groups tested (both human and cattle).

All infections are acquired by the ingestion (or inhalation) of infective oocysts excreted by infected hosts. Most oocysts are fully sporulated and infective when excreted but they are very resistant to a range of environmental conditions. Laboratory studies have shown that oocysts stored in aqueous solutions have remained viable for up to three months at ambient temperature (15-20°C) or for up to one year when refrigerated (4-6°C). Infectivity was lost only after oocysts had been frozen or heated to 65°C for 30 minutes.

Oocysts may be transmitted by direct host-to-host contact or by indirect contamination of the environment, food or water supplies. Many instances of human-to-human transmission have been recorded between household and family members, sexual partners (both heterosexual and homosexual), hospital patients and staff, and children attending day care centres.

Numerous cases of zoonotic transmission from animals to humans have also been inferred from epidemiological studies; the majority involving people caring for pets or farm animals (particularly calves). Zoonotic transmission has been confirmed several times by the accidental infection of animal attendants and research workers maintaining isolates in laboratory animals. Most cases involved oocyst transmission by faecal contamination but

two cases reported the aerosol transmission of oocysts coughed up by animals during their inoculation. Aerosol transmission has previously been suggested for the dissemination of respiratory cryptosporidiosis in birds.

Contaminated water was first implicated as a source of infection among international travellers and several outbreaks have subsequently been associated with the contamination of well water, surface waters and even a swimming pool. *Cryptosporidium* oocysts have since been recovered from untreated surface waters (rivers, streams and reservoirs), untreated and treated sewage effluents, and most importantly, from treated drinking water supplies (despite filtration through sand filters and/or disinfection by chlorination). Recent studies have indicated that most conventional methods of water treatment do not effectively remove or kill all *Cryptosporidium* oocysts from contaminated water. Alternative methods of water treatment must be examined to reduce the potential for the water-borne transmission of cryptosporidiosis.

Several epidemiological studies have also implicated contaminated food in the transmission of infections; especially unpasteurized milk. Although no detailed studies have confirmed this mode of transmission, foodstuffs prepared with contaminated water or grown in soil fertilized with human or animal waste should be regarded as potential sources of infection.

DIAGNOSIS

Cryptosporidium infections were originally diagnosed in animals by the histological examination of tissues collected at autopsy. The first infections recorded in humans were diagnosed in intestinal biopsy material by histological examination and subsequently confirmed by transmission electron microscopy.

Under the light microscope, endogenous developmental stages appear as small spherical bodies attached to the luminal surface of the host epithelial cells. The mucosal brush border frequently has a spotty granular appearance, particularly in heavily infected tissues. Most developmental stages are basophilic and stain well with haematoxylin and eosin or Giemsa stains. Although a variety of other specialized histochemical stains have been examined, they have not produced any marked improvements in staining intensity or resolution.

Transmission electron microscopy has often been used in the past to confirm the diagnosis of infections because little morphological detail was apparent in histological sections. Different endogenous developmental stages of the parasite are evident in the host cell microvillous region as trophozoites, meronts, gametocytes or developing oocysts located within parasitophorous vacuoles.

Autopsy or biopsy tissue samples taken for histological or electron microscopic examination must be fixed as soon as possible after collection or the death of the host to avoid autolytic degeneration and sloughing of the epithelial cells. Fixatives should be perfused into the lumina of infected organs where possible. Fixed tissues should also be processed carefully to avoid the detachment of organisms.

Although the detection of parasites in host tissues provides definitive proof of infection, histological and ultrastructural examination techniques are costly and time-consuming. Only small pieces of tissue are examined and preparative artefacts are common. Infections are not uniformly distributed throughout the tissues and light infections may be overlooked. While such techniques may be used to investigate pathological and cytological changes accompanying infections, they should not be used exclusively for diagnostic purposes.

Indirect methods of diagnosing infections by comparative symptomatology or animal inoculation are also unsuitable for a variety of reasons. If clinical signs of disease are present, they may vary considerably depending on the host species involved and its immune and nutritional status as well as the parasite species (or strain) involved and the severity of infection. Even when present, the clinical signs of disease are very nonspecific (mainly involving diarrhoea) and could be attributable to a variety of other enteropathogenic agents or pathological conditions.

The inoculation of infected host material into laboratory animals has previously been used to confirm the presence of infections but variations in parasite infectivity and host susceptibility may limit the success of parasite transmission. Some parasites have been found to undergo complete cycles of development when inoculated into chicken embryo chorioallantoic membranes. However, parasite development only occurred in embryos derived from certain flocks and not all isolates exhibited good growth characteristics. The reasons for the failure of many chicken embryos to support parasite development are not yet known. More recently, parasites have been successfully grown in tissue culture on certain cell lines but their multiplication and development has been limited compared to that occurring in experimentally infected animals. Further studies are required to establish *in vitro* cultivation techniques for the propagation of *Cryptosporidium* isolates.

At present, most infections are diagnosed by the microscopic examination of host faecal material for the presence of *Cryptosporidium* oocysts. Experimental studies have shown that the excretion of oocysts coincides well with the onset and duration of most clinical signs of disease accompanying acute or chronic infections in immunocompetent or immunocompromised hosts. In an increasing number of cases, oocysts have also been found

to be excreted sporadically after the resolution of all clinical signs of disease. Other body fluids such as bile, sputum or respiratory aspirates may be examined for oocysts if extra-intestinal infections are suspected. Material from asymptomatic individuals can be screened to detect subclinical infections and even water samples can be checked for contamination by oocysts.

Most conventional parasitological techniques used for coprological examinations are not entirely suitable for the detection of *Cryptosporidium* oocysts. The oocysts are much smaller than those of other coccidian parasites and they differ in many of their staining and buoyancy characteristics. Various histochemical staining techniques, concentration procedures and immunolabelling techniques have therefore been developed to facilitate the reliable detection of oocysts even when present in small numbers. Such techniques are now commonly used in clinical microbiology and parasitology laboratories for diagnostic and research purposes.

Table 6. Staining procedures for *Cryptosporidium* oocysts.

Staining technique	Appearance of oocysts	Appearance of yeasts
Direct staining		
Giemsa	blue	blue
methylene blue	light blue	dark blue
aniline-carbol-methyl violet	blue	unstained
safranin + counterstain	orange	counterstained
acid-fast stains		
- Kinyoun	red	counterstained
- Ziehl-Neelsen	red	counterstained
- DMSO-carbol fuchsin	red	counterstained
fluorescent stains		
- auramine-O	yellow	unstained
- acridine	orange	orange
Negative staining		
carbol fuchsin	unstained	blue
periodic acid-Schiff	unstained	red
nigrosin	unstained	unstained
iodine	unstained	yellow
methanamine silver	unstained	black
phosphotungstic acid	light brown	black
uranyl acetate	light brown	black

Various specialized staining procedures have been described to stain the walls and/or contents of mature oocysts. The oocysts are similar in size and shape to other faecal components (especially some yeast cells) therefore differential staining techniques are preferred to avoid confusion. Some expertise in the identification of oocysts is also required because they can exhibit some variability in their staining characteristics depending on their age, viability and stage of development (even though most oocysts are excreted fully sporulated). Both fresh and fixed faecal material can be processed for staining and many laboratories recommend the submission of fixed samples because of biohazard considerations. Acceptable fixatives include 10% formalin and sodium acetate-acetic acid-formalin (SAF) but polyvinyl alcohol (PVA) fixatives are not compatible with most staining procedures.

Although many staining techniques have been described, the method of choice for most diagnostic laboratories has been acid-fast staining. Fixed faecal smears may be stained with basic fuchsin using hot or cold staining procedures. Oocysts stain bright red whereas yeasts, bacteria and other faecal debris only take up the counterstain. Despite the apparent specificity of such staining techniques, their sensitivity may be limited because only a small volume of faecal material is examined and light infections may be overlooked. Various fluorescent stains have also been used to stain oocysts but their identification usually needs to be verified by other staining techniques.

More recently, several immuno-labelling techniques have been developed to detect oocysts. Both polyclonal and monoclonal antibodies raised against oocyst antigens have been used with fluorescent or enzyme labels to detect oocysts in faecal and water samples. Several test kits are now commercially available for diagnostic use. Although the sensitivities of such tests may be high (particularly for samples containing large amounts of debris and few oocysts), the specificities of the antibodies for different parasite species or isolates remains to be determined.

Many laboratories prefer to use various sedimentation or flotation techniques to concentrate and recover oocysts from larger sample volumes to improve test sensitivity. Oocysts have been successfully concentrated by centrifugal sedimentation in formalin-ether and formalin-ethyl acetate solutions or by centrifugal flotation in saturated sucrose, sodium chloride, zinc sulphate, magnesium sulphate and potassium iodide solutions.

Comparative studies on the sensitivities of these concentration techniques have yielded conflicting results. Several studies found few differences between techniques whereas others found sucrose flotation techniques to be the most sensitive. In our experience, *Cryptosporidium* oocysts are less buoyant than those of other coccidian parasites therefore high specific-gravity flotation media (such as potassium iodide) are routinely used in our diagnostic laboratory. In either case, samples must be examined soon after preparation because prolonged exposure to most flotation media results in oocyst degeneration and collapse.

Specialized centrifugation techniques have also been developed to purify oocysts from faecal material for experimental purposes. These techniques have included isopycnic or density gradient centrifugation in caesium chloride, Percoll or Ficoll-sodium diatrizoate solutions. Pure oocyst suspensions can be obtained free of faecal contaminants but such techniques are not suitable for routine diagnostic use due to time and cost constraints.

Faecal concentrates are best examined for oocysts by phase-contrast microscopy at 200-400 times magnification. The oocysts appear as phase-bright, birefringent bodies against a dark background and they usually contain one to several eccentric dark granules (see Fig. 6). In comparison, yeasts and other faecal debris are not phase-bright but remain dull and dark in appearance. When examined by normal bright-field microscopy, the oocysts appear as small, non-refractile bodies which are difficult to detect even though they sometimes appear light pink in colour. Nomarski interference-contrast microscopy has also been found to be most suitable for discerning internal structures within oocysts (i.e. sporozoites and crystalline residual body). The identity of oocysts in faecal concentrates can also be checked by washing them soon after flotation or sedimentation and preparing smears for histochemical staining or immuno-labelling.

Different concentration techniques have been used to recover oocysts from water samples. Basically, they have involved pumping water through spun polypropylene cartridge filters (1 μm pore size), eluting the sediment from the filter and then examining it for oocysts by various staining or immuno-labelling techniques. Numerous problems have been encountered with these filtration techniques (including clogged filters, poor elution, sediment aggregation) but various refinements have been incorporated to improve organism recovery (including pre-filtering, back-flushing, pressure elution, sediment dispersal and re-concentration). Despite the inherent technical problems, oocysts are being detected with alarming frequency in both untreated and treated water supplies.

In the last few years, various laboratories have developed immunoserological tests to detect specific antibodies against *Cryptosporidium* in host serum samples. Tissue sections containing endogenous developmental stages and oocyst preparations have been used as antigens in indirect fluorescent-antibody tests and soluble oocyst extracts have been used in enzyme immunoassays. Antibodies present in test sera are visualized using antisera conjugated to fluorochrome or enzyme labels. However, positive reactions should not be regarded as being indicative of active infection but rather as providing presumptive evidence of prior exposure. Nevertheless, serological studies have indicated that infections may occur more frequently in humans and animals than previously thought.

INFECTIONS IN MAN

Cryptosporidium infections were first diagnosed in two human patients in 1976 and a further eleven cases were reported over the next six years. The parasites were generally thought to be rare opportunistic pathogens as most cases involved immunocompromised individuals. In 1982, infections were associated with significant mortality rates in AIDS patients and the first clinical outbreak was recorded in immunocompetent individuals. Since then, infections have been detected in humans from over 50 different countries located on all inhabited continents. The parasite is now regarded to be a common cause of diarrhoea in humans throughout the world.

Most reports on the prevalence of infections have been made by diagnostic laboratories examining faecal samples from patients experiencing diarrhoea. Excluding reports on AIDS patients and specific outbreak situations, prevalence results have ranged from 0.1-27.1% in developed industrialized countries (mean of 56 surveys = 4.9%) compared to 0.1-31.5% in under-developed countries (mean of 48 surveys = 7.9%). Samples from asymptomatic individuals have also been examined in recent surveys and prevalence reports have ranged from 0-2% in developed countries (mean of 12 surveys = 0.3%) compared to a range of 0-9.8% in less developed countries (mean of 20 surveys = 1.6%). The higher prevalence of infections in less developed countries may be related to poor sanitation, overcrowding, contaminated water supplies or greater contact with domestic animals.

More recently, immunological tests have been used in a limited number of surveys to provide serological evidence of previous exposure to infections. Seroprevalence results have ranged from 25-86% (mean of 7 surveys = 54%). These results suggest a far greater level of exposure to infections than indicated by coprological studies; perhaps because many subclinical or mild cases are not diagnosed.

In clinical infections, the most common feature of cryptosporidiosis is diarrhoea which is profuse and watery and often contains mucus but rarely blood or leucocytes. Bowel motions may be copious and as frequent as 10 or more per day contributing to rapid weight loss. Other clinical signs observed have included crampy abdominal pain, low grade fever, nausea and vomiting. Other nonspecific symptoms such as malaise, weakness/fatigue, headache, myalgia, anorexia and even reactive arthritis have occasionally been reported.

The duration and resolution of clinical signs depends largely on the immune status of the individual. Most immunocompetent persons experience mild transient enteritis which resolves spontaneously within 1-2 weeks whereas most immunocompromised patients experience

severe unremitting diarrhoea which may not resolve and can become life-threatening. However, some variability in the clinical course of infections has been observed between these patient groups. Mild transient infections have been recorded in some immunocompromised patients and prolonged infections have been found in several immunocompetent patients. Nonetheless, individuals most susceptible to symptomatic and prolonged infections are those whose immune function has been impaired or has not fully developed.

Young children (usually < 5 years old) appear to be more susceptible to infections than adolescents or adults presumably due to their immunological immaturity as well as their greater risk of infection due to unhygienic behaviour. There is some evidence to suggest that the prevalence of infections is lower in breast-fed than bottle-fed infants and failure to thrive has tentatively been associated with persistent infections. A variety of other factors have also been suggested to predispose towards symptomatic or prolonged infections; including malnutrition, pregnancy and concomitant viral infections such as measles, chickenpox and cytomegalovirus.

Protracted clinical infections have been reported in many patients undergoing immunosuppressive drug therapy either for the treatment of skin lesions, leukaemia, lymphoma and other forms of cancer or following organ or tissue transplantation. In many instances, infections have subsided or resolved when immunosuppressive therapy was discontinued.

Severe clinical infections have also been detected in patients with congenital immunodeficiencies (hypo- or a-gammaglobulinaemia) or acquired immunodeficiencies (HIV-positive patients who have developed AIDS). In many AIDS patients, infections have produced persistent diarrhoea which worsened with time and eventually contributed to death. Infections are not always confined to the small intestines and parasites have been found in the oesophagus, stomach, appendix, colon and rectum.

Extra-intestinal infections have also been reported with increased frequency over the last decade; mainly in immunocompromised patients. Infections of the gall bladder, liver and pancreas have been associated with cholecystitis, hepatitis and pancreatitis. Respiratory infections have been associated with various clinical signs including coughing, wheezing, croup, hoarseness and shortness of breath. There have been no confirmed cases of disseminated infections in man although parasites have been detected in macrophages in the lungs of one patient and in submucosal blood vessels in the colon of another.

Histopathological changes observed in infected tissues have been relatively nonspecific. Enteric infections have been associated with mild to severe villous atrophy, increased crypt size and inflammatory changes marked by cellular infiltrates in the lamina propria (mainly neutrophils and plasma cells, but sometimes macrophages and lymphocytes). Respiratory infections have been associated with similar cellular infiltration in the subepithelial lamina propria together with deciliation, hyperplasty or hypertrophy of the respiratory epithelium.

A variety of immune mechanisms have been implicated in host resistance or susceptibility to infection, the modulation and eradication of active infection and the acquisition of protection against subsequent infection. Both cellular and humoral immune responses may be involved as evidenced by chronic infections developing in immunocompromised patients with lymphocyte or gamma-globulin deficiencies or abnormalities. Immunological studies have demonstrated specific IgG, IgM, IgA and even IgE antibody responses to infection and *in vitro* studies have indicated that sporozoite infectivity may be neutralized by exposure to immune serum. However, specific antibodies have also been detected in most AIDS patients with chronic infections. It has therefore been suggested that immunological protection may require T-cell induction to provide effective immune responses involving other cellular and humoral mechanisms.

INFECTIONS IN MAMMALS

Although *Cryptosporidium* infections had previously been described in several mammalian species, it was not until 1971 that they were first associated with clinical disease in a calf. Since then, both clinical and subclinical infections have been recorded in 52 different species of mammals (including man). Infections have been detected in six orders of eutherian mammals; including artiodactyls (28 host species), rodents (10 species), primates (5 species), carnivores (4 species), perissodactyls (1 species) and lagomorphs (1 species). Infections have also been found in both orders of marsupials; polyprotodonts (1 dasyurid species) and diprotodonts (1 macropodid species).

Natural infections have been described in domesticated, wild and captive mammals; the majority being reported in farm, zoo, pet and laboratory animals. Although nine *Cryptosporidium* spp. have been described in mammals, only two are currently considered to be valid; *C. parvum* and *C. muris*. Most infections in mammals have been attributed to *C. parvum* which is usually found in the small intestines and has been associated with clinical disease. In contrast, relatively few have been attributed to *C. muris* which has only been described in the gastric mucosa of asymptomatic mice, rats, cattle, mountain gazelles and more recently, experimentally infected cats, rabbits and guinea pigs. Most clinical cases of cryptosporidiosis in mammals are therefore apparently due to *C. parvum*.

Table 7. Mammalian hosts of *Cryptosporidium* spp.

1907	*Mus musculus*	mouse
1912	*Oryctolagus cuniculus*	rabbit
1931	*Apodemus sylvaticus*	field mouse
	Clethrionomys glareolus	red-backed mouse
1966	*Cavia porcellus*	guinea pig
1971	*Bos taurus*	ox
1972	*Macaca mulatta*	rhesus monkey
1974	*Ovis aries*	sheep
1977	*Sus scrofa*	pig
1978	*Antechinus stuartii*	brown antechinus
	Equus caballus	horse
1979	*Felis catus*	cat
1981	*Cervus elaphus*	red deer
	Capra hircus	goat
1982	*Procyon lotor*	raccoon
	Sciurus carolinensis	gray squirrel
1983	*Bubalus bubalis*	water buffalo
	Canis familiaris	dog
	Gazella subgutterosa	Persian gazelle
	Saimiri sciureus	squirrel monkey
1984	*Capreolus capreolus*	roe deer
1985	*Addax nasomaculatus*	addax
1986	*Aepyceros melampus*	impala
	Antilope cervicapra	blackbuck
	Antidorcas marsupialis	springbok
	Boselaphus tragocamelus	nilgai
	Capra falconeri	Turkomen markhor
	Chinchilla laniger	chinchilla
	Cervus axis	axis deer
	Cervus duvauceli	barasingha deer
	Cervus eldi	Eld's deer
	Cervus nippon	sika deer
	Dama dama	fallow deer
	Gazella dama	Addra gazelle
	Gazella leptoceros	gazelle
	Hippotragus niger	sable antelope
	Mesocricetus auratus	golden hamster
	Odocoileus hemionus	mule deer
	Oryx gazella	oryx
	Ovis orientalis	Armenian mouflon
	Rattus norvegicus	rat
	Saguinus oedipus	tamarin
	Taurotragus oryx	eland
	Varecia variegata	red-ruffed lemur
1987	*Ammotragus lervia*	Barbary sheep
	Castor canadensis	beaver
	Gazella gazella	mountain gazelle
	Macaca nemestrina	pig-tailed macaques
1988	*Mustela putorius*	ferret
1989	*Rattus rattus*	rat
1990	*Macropus rufus*	red kangaroo*

* new host record

Young animals appear to be more susceptible to clinical infection and high mortalities have been recorded in neonates of many species; especially ruminants. Outbreaks of disease have been associated with intensive husbandry, seasonal breeding and mixed grazing practises as well as contamination events involving food, water and holding facilities. Most infections detected in adult animals have been asymptomatic or have only been associated with mild clinical signs. However, severe infections have been detected in various immunosuppressed or immunodeficient hosts (mainly dogs, cats, horses and monkeys).

A variety of clinical signs, gross lesions and histopathological changes have been described in infected animals but most have been consistent with acute superficial enteritis. The differential diagnosis of infections may frequently be complicated by the presence of other enteropathogenic agents (such as rotavirus, coronavirus, enterotoxigenic *Escherichia coli*, *Salmonella* spp. or *Campylobacter* spp.). Although it has been suggested that some synergism may occur between these different organisms in concomitant infections with *Cryptosporidium*, this has not been demonstrated experimentally.

The most common feature of cryptosporidiosis in mammals is profuse watery diarrhoea which sometimes appears pale yellow in colour and may have a distinctive offensive smell. Other clinical signs observed have included dehydration, fever, anorexia, weight loss, weakness and progressive loss of condition together with rough coat, depression, dullness and sometimes bloating. Most animals exhibit spontaneous recovery within 1-2 weeks of infection but significant mortalities may occur in young animals.

Post-mortem examination usually reveals little of pathognomonic significance. The intestines may be distended with gas and contain watery fluid (particularly in ruminants) and other nonspecific signs such as congested mucosae, enteritis and colitis may sometimes be observed.

Most histopathological observations have included mild to moderate villous atrophy (incorporating shortened, fused or blunted villi), crypt hyperplasia, some focal necrosis and loss of epithelial cells and light to moderate cellular infiltrates in the lamina propria (predominantly mononuclear cells). The mucosal epithelium may also exhibit some metaplasia to low columnar, cuboidal or even squamous epithelial forms.

In most infections, parasites have only been detected in the small intestines. However, organisms have occasionally been detected in other sites including the caecum, colon, stomach, gall bladder, liver and pancreas of naturally-infected animals and more recently, in the conjunctiva, trachea or uterus of experimentally-infected pigs and mice.

INFECTIONS IN BIRDS

Cryptosporidium infections have been detected in 28 species of birds belonging to six different orders; namely, Psittaciformes (10 host species), Galliformes (7 species), Anseriformes (5 species), Passeriformes (5 species) and Columbiformes (1 species). Most infections have been detected in domestic flocks or aviary birds whereas few infections have been recorded in wild bird populations.

Table 8. Avian hosts of *Cryptosporidium* spp.

1929 *Gallus gallus*	chicken
1955 *Meleagris gallopavo*	turkey
1974 *Anser anser*	goose
1979 *Amazona autumnalis*	red-lored parrot
1980 *Pavo cristatus*	peacock
1982 *Coturnix coturnix*	common quail
1983 *Anas* sp.	duck
Serinus canarius	canary
1984 *Poephila cincta*	black-throated finch
1985 *Phasianus colchicus*	pheasant
1986 *Colinus virginianus*	bobwhite quail
Gallus sonneratti	junglefowl
1987 *Agapornis* sp.	lovebird
Anas platyrhynchos	Mallard duck
Cairina moschata	Muscovy duck
Melopsittacus undulatus	budgerigar
1988 *Ara* sp.	macaw
Carduelis carduelis	goldfinch
Columba livia	pigeon
Cygnus sp.	Tundra swan
un-named species	Nymphensittich?
un-named species	Plattschweifsittich?
un-named species	Strohsittich?
un-named species	Grassittich?
un-named species	Sperlingspapagei?
un-named species	Schwarzohrpapagei?
1989 *Nymphocus hollandicus*	cockatiel
1990 *Staganoplura bella*	diamond firetail finch

Cryptosporidiosis in birds is usually manifest as respiratory or enteric disease, although both have sometimes been reported together. Renal disease has also been described on several occasions. Many case reports have involved acute clinical disease and high mortalities have been recorded in commercial flocks (mainly chickens, turkeys, pheasant and quail). Young birds appear to be the most susceptible to clinical infections.

At present, two *Cryptosporidium* spp. in birds are considered to be valid (*C. meleagridis* and *C. baileyi*) but most reports of natural infections have not provided enough information to determine which species was involved. Studies performed on experimentally-infected birds have suggested that *C. meleagridis* may be involved in most enteric infections whereas *C. baileyi* may be associated with most respiratory infections. However, neither species is strictly confined to these different sites of infection (each has been found in both locations). Individual parasite species therefore cannot be identified with certainty based on the site of infection nor by inference, on the basis of any clinical signs accompanying infection. Previous reports have indicated that respiratory infections are more common and widespread in birds than enteric infections.

Clinical signs associated with respiratory infections in different bird species have generally been similar and involved rales, coughing, convulsive sneezing, mucoid discharges and dyspnoea. Post-mortem examination usually reveals excess mucus and sometimes petechiae in the trachea and nasal cavities and airsacculitis has occasionally been observed. Histo-pathological changes have included hypertrophy, hyperplasia and deciliation of the respiratory epithelium with mononuclear cell infiltration of the interstitial tissue and occasionally the epithelium itself.

Parasites have been detected in many locations throughout the respiratory tract including the nasopharynx, larynx, trachea, primary and secondary bronchi and air sacs. Natural and experimental infections of the conjunctiva have also been reported in association with conjunctivitis.

Enteric infections have been associated with mild to severe watery diarrhoea, dehydration, depression, weight loss and weakness. Post-mortem examination has usually revealed the small intestines (and sometimes the caecum) to be thin-walled and distended with gas and cloudy mucoid material or watery fluid. Various histopathological changes have been reported including villous atrophy and fusion, epithelial hyperplasia and hypertrophy, crypt hyperplasia, bursal follicular atrophy and mild to moderate cellular infiltration of the lamina propria.

Parasites have usually been found in the ileum, caecum, colon, cloaca and bursa although they have occasionally been detected in the proventriculus, salivary glands and oesophageal glands. Experimental infections have also been established in the gall bladder and associated with epithelial hyperplasia and infiltration of the connective tissues with mononuclear cells.

Renal infections have only been detected at necropsy in finches, jungle fowl and chickens. Grossly, the kidneys were pale and enlarged, sometimes with white foci in the parenchyma. Histopathological changes observed included epithelial hypertrophy and hyperplasia in the collecting tubules, convoluted tubules and ureters with cellular infiltration in the interstitial tissues. Parasites were detected in the collecting ducts and convoluted tubules.

INFECTIONS IN REPTILES

The first four reports of *Cryptosporidium* spp. in reptiles made between 1925 and 1969 are no longer considered to be valid because the descriptions of the parasites detected are more consistent with those of *Sarcocystis* sporocysts than *Cryptosporidium* oocysts. The first valid reports of *Cryptosporidium* infections in reptiles were made in 1977. Infections have since been reported in 36 different reptilian species; including 25 species of snakes (boids, colubrids, elapids and viperids), 9 species of lizards (agamids, gekkonids and chamaeleonids) and 2 species of tortoises (both testudinids). Most infections have been detected in captive animals whereas they have only rarely been encountered in wild-caught reptiles.

Table 9. Reptilian hosts of *Cryptosporidium* spp.

1977	*Crotalus horridus*	rattlesnake
	Elaphe guttata	corn snake
	Elaphe obsoleta	rat snake
	Elaphe subocularis	rat snake
	Sansinia madagascarensis	Madagascar boa
1978	*Pseudechis porphyriacus*	red-bellied black snake
1983	*Chameleo senegalensis*	chameleon
1985	*Bitis gabonica*	Gaboon viper
	Oxyuranus scutellatus	taipan
1986	*Crotalus* spp.	rattlesnakes
	Geochelone elegans	star tortoise
	Pituophis melanoleucus	pine snake
1987	*Bothrops nummifer*	viper
	Crotalus cerastes	rattlesnake
	Phelsuma madagascariensis	gecko
	Sistrurus catenatus	snake
	Thamnophis sirtalis	garter snake
1988	*Corallus caninus*	emerald tree boa
	Geochelone carbonaria	red-footed tortoise
	Lichanura trivirgata	rosey boa
1989	*Agama aculeata*	lizard
	Agama planiceps	Damara rock agama
	Chondrodactylus angulifer	gecko
	Constrictor constrictor	boa constrictor
	Crotalus durissus	rattlesnake
	Elaphe longissima	rat snake
	Elaphe quatuorlineata	rat snake
	Elaphe schrencki	rat snake
	Elaphe vulpina	fox snake
	Hemidactylus turcicus	gecko
	Lacerta sp.	lizard
	Lampropeltis triangulum	milk snake
	Naultinus grevi	lizard
	Nerodia harteri	water snake
	Nerodia rhombifera	water snake
1990	*Agama stellio*	starred lizard

Over the last 2 years, our laboratories have recorded infections in a further 9 species of snakes (boids and elapids) and another lizard species (helodermatid). All infections were detected in captive animals. However, a small scale survey performed on wild-caught reptiles has revealed infections in several red-bellied black snakes from two separate geographic locations in South Australia.

Table. 10. New reptilian hosts for *Cryptosporidium* spp.

1991	*Acanthophis antarticus*	death adder
	Aspidites ramsayi	Woma python
	Heloderma suspectum	Gila monster
	Morelia spilota	carpet python
	Notechis ater	tiger snake
	Oxyuranus microlepidotus	inland taipan
	Pseudechis guttatus	spotted black snake
	Pseudonaja affinis	dugite
	Pseudonaja nuchalis	Western brown snake
	Pseudonaja textilis	common brown snake

The clinical course of cryptosporidiosis in snakes is markedly different from that in mammals and birds. Nearly all infections in snakes have been associated with chronic gastric disease as opposed to the acute enteric or respiratory diseases found in mammals and birds. Most infections have also been detected in mature snakes whereas they are more commonly found in neonatal and adolescent mammals and birds. Infections may also persist for some time and intermittent oocyst excretion has been recorded in individual snakes for periods ranging from several months up to two years.

Clinical signs frequently observed include anorexia, progressive weight loss, postprandial regurgitation and firm midbody swelling. Protracted clinical disease may eventually result in host death. In many instances, post-mortem examination reveals marked thickening of the stomach wall with considerable narrowing of the lumen (often severe enough to prevent the entry of ingesta). Petechial haemorrhages, excess mucus production and exaggeration of the longitudinal rugae have also been observed. Endogenous developmental stages of the parasite have only been detected in association with the gastric mucosa in snakes. Histopathological changes observed have included inflammation, hyperplasia and hypertrophy of the gastric glands and oedema of the submucosa and lamina propria with cellular infiltrates.

Most cases reported in lizards have involved subclinical gastric infections. However, some have been associated with clinical signs involving anorexia, weight loss and lethargy. More recently, parasites were also detected in an unusual location in the cloaca of several moribund geckos but their clinical significance was not determined.

Infections have only been recorded in tortoises on two separate occasions. One report described clinical signs including gastritis and regurgitation whereas the other described progressive wasting.

Recent studies have indicated that multiple parasite species may occur in reptiles. Morphometric analyses of oocysts recovered from snakes and lizards have revealed at least five different morphological types. One type conforms to previous descriptions of *C. serpentis* but it is not known whether the remainder represent additional parasite species. Several studies have also presented circumstantial evidence suggesting that captive snakes may have contracted infections through the consumption of infected prey animals (such as laboratory mice). However, a recent study failed to transmit a *C. serpentis* isolate to mice and the electrophoretic protein profiles of *C. serpentis* and *C. parvum* oocysts were found to exhibit marked differences. Nonetheless, the taxonomic status and host specificities of different isolates from reptiles remains to be determined.

INFECTIONS IN FISH

Cryptosporidium infections have been detected in six species of fish, including marine and freshwater species. Most infections have been detected in cultured, captive or ornamental fish species but oocysts have recently been recovered from wild-caught fish.

Table 11. Piscine hosts of *Cryptosporidium* spp.

1981 *Naso literatus*	naso tang
un-named species	marine tropical fish
1983 *Cyprinus carpio*	carp
1986 *Oreochromis* hybrids	cichlids
1987 *Lates calcarifer*	barramundi
Salmo trutta	brown trout

Most infections in fish have not been associated with clinical signs of disease except for the original report which described a progressive illness in a single naso tang characterized by anorexia, emaciation, regurgitation and the passage of faeces containing undigested food. Parasite developmental stages were found attached to the intestinal mucosa but no pathological changes were evident. Subsequent studies have detected parasites in the intestines of all other fish species except cichlids where they were confined to the stomach mucosa. Few histopathological changes have been described although some cellular infiltrates were observed in the lamina propria of the small intestines of barramundi and small pockets of necrotic epithelial cells containing oocysts were detected in the stomach mucosa of cichlids.

INFECTIONS IN OTHER HOSTS

All previous reports of *Cryptosporidium* infections have been confined to hosts belonging to four vertebrate classes; mammals, birds, reptiles and fish. However, several studies have reported infections in other host species.

Infections have been detected in amphibian hosts on two occasions. In 1987, organisms were recorded from a single frog (*Ceratophrys ornata*) at the Toronto Zoo but detailed information was not given. At the beginning of 1992, our laboratories detected oocysts in the faeces of a spotted grass frog (*Limnodynastes tasmaniensis*) collected in South Australia. Although oocysts were relatively numerous in the faeces, no clinical signs of disease were evident. The oocysts were similar in size, shape and appearance to those of *C. serpentis* previously described in snakes but it is not known whether they represent the same species.

Parasites presumed to be *Cryptosporidium* have also been described in an invertebrate host. In 1989, asexual developmental stages of a parasite were described in the gill epithelia of the Portugese clam *Ruditapes decussatus* (bivalve mollusc). Meronts containing up to 50 merozoites were found enclosed within large parasitophorous vacuoles and several merozoites were found to be attached to the inner parasitophorous vacuole membrane by knob-like organelles. However, characteristic attachment organelles were not observed and oocysts and other life cycle stages were not detected. The merozoites possessed many organelles typical of apicomplexan parasites but they differed from *Cryptosporidium* merozoites in that they contained prominent rhoptries and a distinct conoid. The identification of these parasites as *Cryptosporidium* therefore seems questionable.

TREATMENT AND CONTROL

At present, there is no effective treatment available for cryptosporidiosis in man or animals.

Many infections in immunocompetent hosts appear to be asymptomatic or only associated with mild clinical signs which resolve within 1-2 weeks. However, some cases may be severe enough to warrant medical or veterinary intervention. Supportive care with oral or intravenous fluid replacement has been found to be beneficial primarily in alleviating the dehydration accompanying acute diarrhoea while awaiting spontaneous recovery. Parenteral nutrition may sometimes be deemed necessary and antidiarrhoeal compounds may assist in controlling fluid loss.

However, both acute and chronic infections have been associated with severe clinical disease and mortalities in various host species (particularly neonatal animals and immuno-compromised humans) despite the provision of

supportive care. Some form of effective treatment is urgently required. To date, over 100 different compounds have been tested against infections without marked success although several preparations have recently been reported to have exhibited some therapeutic effect.

Table 12. Substances tested against *Cryptosporidium*

Alborixin*	Lasalocid*
Amikacin	Levamisole
Amphotericin B	Lincomycin
Ampicillin	Loperamide
Amprolium*	Maduramycin*
Arprinocid*	Mebendazole
Azidothymidine*	Mepacrine
Azithromycin*	Methylbenzoquate
Bismuth subsalicylate	Metronidazole
Bleomycin	Monensin
Bovine leucocyte extract*	Morphine sulphate*
Bovine colostrum	Naproxyn
Carbenicillin	Neomycin
Cefamandole	Nicarbazin
Chloramphenicol	Nitrofurantoin
Chloroquine	Nystatin
Cholestyramine	Octreotide
Cimetadine	Oxytetracycline
Clindamycin*	Paromomycin
Clonidine	Penicillin
Clopidol	Pentamidine
Cloxacillin	Phenamidine
Colistin	Piperazine
Cotrimoxazole	Polymixin
Cyclosporin A*	Primaquine
Decoquinate	Pyrimethamine
Diclazuril	Quinacrine
Di-iodohydroxy-equinoline	Quinine*
Difluoromethyl-ornithine	Rifampicin
Diloxanide furoate*	Robenidine
Dimetridazole	Salinomycin*
Dinitolmide*	Septrin
Diphenoxylate HCl*	Somatostatin*
Doxycycline	Spiramycin*
Emtryl	Streptomycin
Enterolyte-N	Sulfonamides
Erythromycin*	(Sulfadiazine
Ethopabate	Sulfadimethoxine*
Framycetin	Sulfadimidine
Furaltadone	Sulfamethazine
Furazolidone*	Sulfamethoxazole
Gamma-globulin	Sulfaquinoxaline*)
Gentamicin	Synthetic lytic peptides*
Gluten-free diet	Tetracycline
Halofuginone	Thiabendazole
Hyperimmune bovine colostrum*	Trimethoprim
Indomethacin	Toltrazuril
Interleukin-2*	Tincture of opium
Iodoquinol	Trinamide
Ipronidazole	Vancomycin
Ivermectin	Zidovudine*
Kaolin-pectin	Zoaquin
Ketoconazole	

* = some action or clinical improvement indicated

Anecdotal success has been reported in the treatment of infections in immunocompromised humans with diloxanide furoate, furazolidone, interleukin-2, quinine plus clindamycin or spiramycin. Treatment allegedly resulted in the clinical resolution or eradication of infections but these findings have not been substantiated by subsequent studies. Several controlled clinical trials have recently been performed using spiramycin but the results have not been conclusive. Some symptomatic improvement has also been reported following treatment with azidothymidine, diphenoxylate, erythromycin, morphine sulphate, somatostatin and zidovudine.

Various drugs have also been tested in experimentally infected animals (calves, suckling mice or immunosuppressed rats). Prophylactic treatment with alborixin, amprolium, arprinocid, azithromycin, cyclosporin A, dinitolmide, lasalocid, maduramycin, salinomycin, sulfadimethoxine or sulfaquinoxaline has been reported to restrict parasite development and limit the severity of infections as compared to untreated controls.

Several novel treatment strategies have recently been examined with encouraging results. *In vitro* studies have revealed significant reductions in sporozoite viability following exposure to two synthetic lytic peptides, hecate-1 (melittin-like) and shiva-10 (cecropin-like), which are similar in structure to natural antimicrobial peptides isolated from frogs and silk moths. Sporozoite viability was also reported to be significantly reduced following exposure to hyperimmune bovine colostrum harvested from dairy cows immunized during pregnancy with concentrated oocyst/sporozoite preparations.

Preliminary *in vivo* studies have reported that the prophylactic treatment of mice and calves with hyperimmune colostrum partially protected them against experimental infections and clinical disease. Several immunodeficient patients treated with hyperimmune colostrum have also exhibited symptomatic improvement or even complete recovery from chronic infections. Similar results have also been reported when immunodeficient patients were treated with an uncharacterized dialyzable leucocyte extract prepared from the lymph nodes of infected calves.

It has recently been demonstrated that the immunoglobulin fraction purified from hyperimmune colostrum exhibited a therapeutic effect on infections in mice. Antibodies present in the hyperimmune colostrum have also been found to react with over 40 oocyst/sporozoite antigens in electrophoretic immunoblot studies. Further studies are required to identify the key components involved in protection and determine their modes of action in the treatment of clinical infections in both man and animals.

Various recommendations have been made for the prevention and control of infections in specific locations; such as hospitals, laboratories, day care centres, households, zoos, farms, etc. Such recommendations have basically involved managerial practices designed to minimize further host contact with sources of infections and the use of effective disinfection procedures to destroy infective oocysts.

High standards of hygiene must be observed as applies for other infectious enteropathogens. If practicable, infected individuals should be identified and isolated to confine infections to particular areas. Susceptible individuals should avoid contact with contaminated areas or be removed to safe locations. Care should be exercised in the handling and disposal of biohazardous waste. Suspect contaminated water should be boiled prior to use or consumption. In short, common sense should dictate appropriate precautionary measures.

The disinfection of contaminated areas and materials has proven difficult. Laboratory studies have shown that many disinfectants are ineffective against *Cryptosporidium* oocysts when used at concentrations recommended by the manufacturer. Higher concentrations and relatively long exposure times are required to kill oocysts. To date, over 35 disinfectants have been tested but only six have been found to be effective; 10% formalin, 50% ammonia, 3% (10 vol.) hydrogen peroxide, Exspor (a chlorine dioxide-based sterilant), Oo-cide (a two phase product producing ammonia) and 1 ppm ozone (used for water treatment). Caution is advised when using concentrated disinfectants in confined areas. Steam heat sterilization and fumigation with formaldehyde or ammonia gas have also been recommended as appropriate forms of decontamination.

MOLECULAR BIOLOGY

Over the last five years, several laboratories have used modern molecular biological techniques to develop specific immunoreagents for diagnostic use, to search for parasite antigens involved in the development of protective immune responses and to examine inter- and intra-specific parasite variation.

Monoclonal antibodies have been raised against parasite antigens located on the outer oocyst wall and incorporated into fluorescent antibody tests and enzyme immunoassays for the detection of oocysts in faecal and water samples. However, further studies must be performed to determine the specificities of the monoclonal antibodies for oocysts isolated from different host species. Most monoclonal antibodies have reacted against oocysts recovered from mammalian hosts but some have failed to react, or reacted differently, with those from avian or reptilian hosts.

Many common and unique proteins have been detected in isolates from different mammalian, avian and reptilian hosts by polyacrylamide gel electrophoresis and two-dimensional gel electrophoresis. A range of antigens have also been identified in Western blot studies using host immune sera, polyclonal rabbit antisera, mouse monoclonal antibodies and even hyperimmune bovine colostrum. Most reports have described several immunodominant antigens common to many isolates (especially in the 20,000-35,000 molecular weight range) and studies are currently examining their immunogenic potential. Despite varying degrees of heterogeneity observed in the antibody responses of individual hosts, some unique antigens were evident in several different parasite isolates.

More recently, the chromosomal DNA banding patterns of several isolates from mammals and one from birds were examined by field inversion gel electrophoresis. No differences were detected between isolates from mammals whereas the mammalian isolates were different from the bird isolate.

Despite some evidence to the contrary, most molecular biological studies have tended to support the concept of different *Cryptosporidium* spp. occurring in different vertebrate classes. However, our laboratories have recently used isoenzyme electrophoretic techniques to demonstrate genetic diversity occurring between isolates not only from different vertebrate classes but also within the same vertebrate class. A total of 21 isolates from different mammals, reptiles and birds were examined at 17 different enzyme loci. Seven distinct genetic groups were identified; four in mammals, two in reptiles and one in birds. In mammals, two groups were confined to human patients, one to calves and one to goats. These results suggest that *Cryptosporidium* spp. cannot be classified on the basis of host vertebrate class or oocyst morphology.

In conclusion, there is growing evidence that considerable morphological, biological and molecular variation occurs between different parasite isolates beyond what are currently perceived to be separate species. The present taxonomic classification of *Cryptosporidium* spp. should therefore be regarded as tentative until the significance of such parasite variation can be determined. Recent case reports and cross-transmission studies have also indicated that infections may be transmitted between different vertebrate classes. All isolates should therefore be regarded as potentially zoonotic until proven otherwise.

RECOMMENDED READING

Dubey, J.P., Speer, C.A. and Fayer, R. (Eds). 1990. *Cryptosporidiosis of man and animals*. CRC Press, Boston, U.S.A. 199pp.